REPEATED ZOOSPORE EMERGENCE IN DICTYUCHUS[1]

WILLIAM H. WESTON

(WITH PLATE XXIII AND ONE FIGURE)

Dictyuchus, one of the less known genera of the Saprolegniaceae, was established in 1869 by LEITGEB (6) to include a single species, *D. monosporus;* and in 1872 LINDSTEDT (7) added the two species *D. polysporus* and *D. Magnusii;* while in 1893 a fourth species, *D. carpophorus*, was described by ZOPF (12). LEITGEB observed that in his type species laterally biciliate zoospores emerged from sporangiospores which were invariably retained *in situ*, and that these zoospores swarmed but once; and since this condition has been found in all the other species, it has been regarded as characteristic of the genus.

In this paper certain observations on an undetermined species of *Dictyuchus* are presented, which show that, in this instance at least, the usual history, as described by LEITGEB and others, may be modified through the presence of a second swarming of laterally biciliate zoospores, which occurs after the first has been completed; and since, so far as the writer is aware, a diplanetic condition of this type has not hitherto been noticed in any of the Saprolegniaceae, a record of its occurrence has seemed desirable, even before a comprehensive study of the fungus has been completed.

The *Dictyuchus* in question appeared in a culture of moist sand, leaves, and other débris taken from a shaded brook bed in a ravine near Great Barrington, Massachusetts; and for over a year and a half it has been kept under observation both in gross and pure cultures, which have been subjected to a variety of cultural conditions. During this period, however, all attempts to induce the formation of sexual organs have been without result, although sporangia were readily and abundantly produced, and it is thus impossible to reach any definite conclusion as to its specific identity. That it cannot be referred to *D. carpophorus* Zopf is evident

[1] Contribution from the Cryptogamic Laboratories of Harvard University, no. 85.

from the quite different form of its sporangia, as well as from the absence of the peculiar tubercles which, according to ZOPF, are characteristic of that form. On the other hand, it is probable, as has been suggested by VON MINDEN (8) and FISCHER (4), that the sexual organs which LINDSTEDT found associated with his *D. polyspora*, and on which he based the species, belonged in reality to a member of some other genus which had accidentally been introduced into his cultures. For this reason the species is perhaps best regarded as in all probability invalid; and there thus remain but two others, *D. Magnusii* Lindst. and *D. monosporus* Leitg., with which the present form may be compared. On a basis of sporangial characters alone it might readily be referred to either of these species, and its failure to produce sexual organs may be due to the fact that it is the antheridial strain of a unisexual (dioecious) type, similar to that which both of the last mentioned species are said to illustrate. On the other hand, it may prove to be a neutral strain, comparable with PIETERS' (9) "*Saprolegnia* no. 66" and the undetermined species of *Achlya* studied by the author (11), having lost its ability to reproduce sexually, at least under ordinary conditions. That this may be the correct explanation is further suggested by the fact that such neutral or non-sexual conditions of *Dictyuchus* have previously been reported by HUMPHREY (5), TIESENHAUSEN (10), and others; while VON MINDEN even definitely identifies a form of this nature with *D. monosporus* Leitg. Although it is quite possible that a similar disposition of the present species might prove to be the correct one, a definite specific reference does not seem justified at the present time.

In order to follow its development in detail, cultures which were known to be uncontaminated by other forms were washed repeatedly in sterile water and placed in a drop on a slide. As soon as the zoospores had emerged, swarmed, and come to rest, they were picked up with a capillary pipette, placed in a few cubic centimeters of sterile water, and sprayed by means of an atomizer on nutrient media contained in Petri dishes. The latter were then examined under a low magnification, the positions of single isolated spores were marked, and, after two or three days'

growth, transfers to stock cultures were made from such mycelia as proved to be uncontaminated. The development of the fungus thus isolated was studied for over a year in Van Tieghem cells, Petri dishes, and battery jars, under a great variety of cultural conditions.

The mycelium in its morphological characteristics is very similar to that of other species of Saprolegniaceae which have been grown in pure culture. Physiologically, however, it is characterized by weak growth, and in consequence requires more frequent transfer and more concentrated nutriment for successful maintenance. The process of sporangium formation in its early stages closely resembles that which is usually found in other members of the family. In the young sporangium initials, filled with dense protoplasm, hyaline clefts arise, extend, and divide the contents into subequal, polygonal spore initials. The sudden shrinking of the sporangium with a concomitant vacuolation of the spore initials now takes place; but the vacuolate condition is more persistent than in other genera, since one or two vacuoles are often retained in the spores at maturity.

In the succeeding stages of its development, however, the sporangium shows itself to be quite different from any other member of the family, save perhaps that of the doubtful genus *Aplanes*. During the swelling of the individual sporangiospores which marks the final stage of development of the sporangium, it becomes apparent that the delicate membrane surrounding each spore has become firmly united, not only to the walls of the adjacent spores, but also to the inner surface of the sporangial membrane. This close union of the walls appears to be a fundamental peculiarity in sporangia of the generic type, and sharply distinguishes it from the abnormal but superficially similar conditions which are occasionally encountered in *Saprolegnia*, *Achlya*, and *Thraustotheca*, when large numbers of spores have failed for some reason to make their escape. We are probably justified, moreover, in accepting HUMPHREY'S (**5**, p. 81) tentative suggestion that this characteristic obtains throughout the genus. Study of the empty sporangium alone (fig. 1) might lead one to interpret its so-called "cell-net" structure as the result of a simple division

into cells, rather than of a process of progressive cleavage; but continuous observation of the successive stages of spore formation affords no evidence in support of this assumption. Moreover, in starved sporangia only partially filled with protoplasm the spores when formed are more separate, and on swelling do not become closely pressed together. Consequently, only an incomplete union of the walls takes place (fig. 2); and the spores become rounded off to a greater degree, so that the resultant structure is more easily understood than the "cell-net" condition in the densely filled sporangia. It is of interest to note that ZOPF (12, *pl. 3, fig. 11*) also figures a similar condition in *D. carpophorus*. This union of the walls in the sporangium increases the mechanical strength of the structure; and in consequence the final swelling of the spores is resisted, and they do not burst out of the sporangium, even though it becomes swollen and bulged. Frequently, however, the swelling of the spores, combined with the outward bulging of the terminal wall of the sporangiophore, is sufficient to rupture the sporangial wall at the base (fig. 19), and the sporangium is abjointed as a whole. This occurs quite commonly even in vigorous cultures, as VON MINDEN (8) has observed, and does not appear to be the result of degeneration and senescence, as LEITGEB (6) and FISCHER (4) have stated.

Renewal of the sporangia is effected by cymose branching (fig. 2), and by their formation in basipetal succession (fig. 1). The first method has been regarded as characteristic of *D. monosporus*, and the second as typical of *D. Magnusii;* but the regular occurrence of both methods in our form and even in *D. Magnusii*, according to VON MINDEN, would indicate that these characters are not specific.

In their subsequent development the sporangiospores within the indehiscent *Dictyuchus* sporangium may either emit zoospores or give rise to hyphae of germination. Since LEITGEB'S original description of these processes is quite detailed, only additional or significant points need be mentioned here. The emergence of the zoospore from the sporangiospore, although described at length by this author, is only scantily figured. The accompanying drawings (figs. 3–9), therefore, have been made to illustrate this process.

After the zoospores (figs. 8, 9) have emerged, their general structure and the disposition of the cilia are seen to be very similar to the "secondary" laterally biciliate type in other genera of the family, although these zoospores are longer and more tapering than those of *Achlya* and *Thraustotheca*, while more flattened than those of *Saprolegnia*. On an average the zoospores of our form are about 13 μ long and 10 μ wide. After an active period of variable duration they come to rest, lose their cilia, and encyst, forming spherical, coarsely granular spores which, to avoid confusion, will be called "cystospores" (fig. 10). After a time these may germinate by sending out hyphae in a perfectly normal manner (figs. 16, 17).

When the sporangiospores give rise not to zoospores but to hyphae of germination, the latter push through the enveloping sporangium wall and grow out into the water (fig. 18) in a manner quite similar to that which is said to be characteristic of *Aplanes*. It is to be noted, however, that in *Dictyuchus*, as well as in other genera in which it occurs as an abnormal method of development, such germination only takes place in the presence of nutrient substances, or of such non-nutrient materials as prevent zoospore emergence.

It is clear that the cycle of non-sexual spore formation just described agrees entirely with the usual accounts. In the form under discussion, however, an additional zoospore emergence may take place. This phenomenon was repeatedly observed under the following conditions: A piece of agar covered with mycelium, when transferred from a stock culture to dilute beef extract, rapidly grows to a small compact tuft of hyphae. If this tuft, after a thorough washing to remove the adhering nutriment, is placed in a hanging drop for study, sporangium formation rapidly takes place, and from the sporangiospores large numbers of zoospores emerge and swim about. Finally they come to rest and encyst for the most part along the edge of the drop. After a time some of the encysted spores so situated germinate by hyphae. Many, however, emit zoospores, with the result that all along the edge of the drop may be seen empty cystospores and zoospores

in various stages of emergence. This process, as shown in figs. 10–13, closely resembles the previous emergence of the zoospore from the retained sporangiospores. The zoospore emerging from the cystospore moreover is exactly like the original zoospore which emerged from the sporangiospore. The cultures in which this additional emergence of the zoospore was observed were derived from single spores, and their purity was beyond question.

To appreciate the significance of this repeated zoospore emergence in *Dictyuchus*, it is necessary to consider briefly the corresponding phenomena in certain related types. The genera of the Saprolegniaceae, as is well known, are distinguished by the characteristic peculiarities of their non-sexual reproduction, since a regular and distinct cycle of non-sexual spore production marks each separate genus. It has been customary to arrange the genera in a series in accordance with the degree of simplicity or the complexity of their cycles. Disregarding the question whether such a series represents the elaboration of a simple type or the simplification of a complex one, we may first consider *Saprolegnia*, which has the most extensive cycle. Since in the other main genera the cycles are less extensive in increasing degrees, we may, in accordance with this, arrange a convenient series: *Saprolegnia*, *Achlya*, *Thraustotheca*, *Dictyuchus*, and *Aplanes* (text fig. 1).

The characteristic cycles of non-sexual reproduction in these 5 genera are shown in the accompanying diagrams, which follow the accepted descriptions for all but *Thraustotheca*. In this genus the author has found that the non-motile sporangiospores swell and escape by bursting the enveloping sporangium wall, after which they closely resemble the escaped sporangiospores of *Achlya* in their further development. The genera form a series representing a gradual decrease in the extent of the cycle of non-sexual spore formation. The series ranges from *Saprolegnia*, with its swarming of primary and secondary zoospores, through successive stages to *Aplanes*, which is believed to lack both of these phases of zoospore activity.

In the repeated emergence of its zoospores this species of *Dictyuchus* shows a distinct departure from the cycle of spore formation

that is customarily ascribed to the genus, nor has this phenomenon been reported in the case of other members of the family. In *Pythium*, however, which is more or less closely connected with the Saprolegniaceae, according as one or another of the several theories of relationship is accepted, a similar double swarming of laterally biciliate zoospores has been reported. CORNU (3) in 1872 first noted the fact as follows: "les zoospores dans tous ces genres

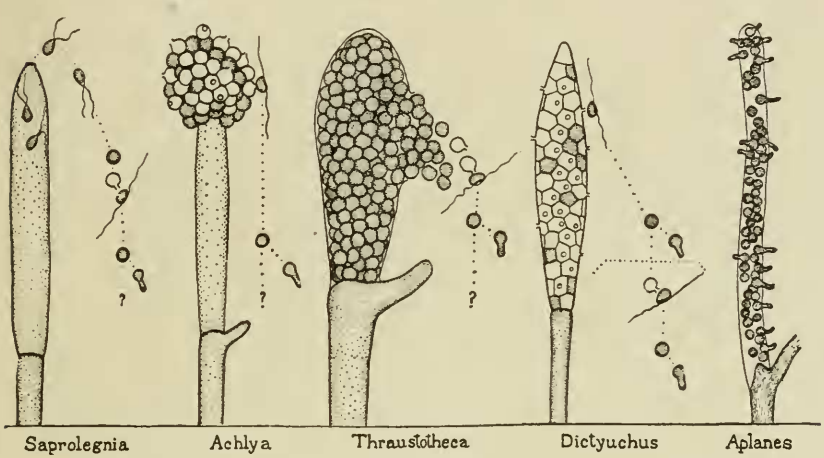

FIG. 1.—Comparative view of cycles of non-sexual spore formation in main genera of Saprolegniaceae; diagrams, of approximately same scale, from drawings of living material, except in case of *Aplanes*, which is after DEBARY'S figure.

germent en donnant lieu à un filament ... ou bien elles émittent des zoospores semblables à elles-mêmes (ex. *Pythium proliferum* et ses var.)." Recently BUTLER (2) has corroborated CORNU'S observations by describing and figuring the process in the case of *Pythium diacarpum*. In spite of the apparent rarity of this phenomenon, however, the writer ventures the opinion that further investigations will bring to light other cases, not only in *Dictyuchus*, but also in related genera of the Saprolegniaceae.

The significance of this repeated zoospore emergence in *Dictyuchus* is a matter of some interest. One may, of course, regard it as a regular but hitherto unobserved stage of the life cycles of

the genus; but in view of the detailed investigations of LEITGEB and others it seems doubtful if such is the case. It must be admitted, however, that *Saprolegnia* had been studied for many years before LEITGEB observed in it the diplanetism on which he based his genus "*Diplanes*." On the other hand, the repeated zoospore emergence in the present instance might be regarded as peculiar to this particular and perhaps hitherto unnoticed species of *Dictyuchus*. Since such an assumption is contrary to our customary conception of the fixity of generic characteristics, its truth may well be doubted. In view of the tardy recognition of the extent of this phenomenon in the genus *Pythium*, it is possible that it has merely been overlooked in the recognized species of *Dictyuchus*. Finally, it is possible that this phenomenon occurs, not only in various species of *Dictyuchus*, but also in other genera of the Saprolegniaceae under certain favorable conditions; and that these conditions either have not been attained in cultures heretofore, or the emergence has escaped observation. It seems highly probable that this is the case, and that in certain Saprolegniaceae there inheres in the protoplasm, even of encysted zoospores of the second type, the ability to form not only germ tubes but also zoospores; and that under proper circumstances the latter may be produced. Probably, then, as ATKINSON (1) has suggested in the case of *Pythium*, this repeated zoospore emergence may best be regarded as a phenomenon of germination, and one which the author believes can be brought about by certain favorable conditions. It is possible that extensive cultural studies of various Saprolegniaceae, with this end in view, will demonstrate that its occurrence is far more widespread than has been suspected. The writer is obliged to admit, however, that he has been unsuccessful in many attempts to induce repeated zoospore emergence in *Achlya* and *Thraustotheca;* but the well known sensitiveness of the Saprolegniaceae to surrounding conditions makes it possible that these failures may have been the result of faulty methods.

In any case, the occurrence of this phenomenon in even a single species of *Dictyuchus* points to the conclusion that the customary application of such terms as "monoplanetic" and "di-

planetic" may be somewhat misleading, and necessitates a modification of our conception of the condition of monoplanetism and diplanetism in the Saprolegniaceae.

Summary

1. In the characteristics of its non-sexual reproduction the fungus which is the subject of this paper shows itself to be a member of the genus *Dictyuchus*. No sexual reproduction was observed, however; hence it cannot be assigned to any of the recognized species.

2. During the formation of spores within the sporangium, the walls of adjacent spores unite with one another and with the enveloping sporangium membrane, to form a polygonally chambered, indehiscent structure. In this respect *Dictyuchus* differs fundamentally from all other Saprolegniaceae, save perhaps the doubtful genus *Aplanes*.

3. The zoospores which emerge from the sporangiospores come to rest and encyst as is customarily described. From these encysted spores in turn, however, laterally biciliate zoospores may emerge. This repeated emergence of laterally biciliate zoospores has not previously been reported in any member of the Saprolegniaceae.

4. It is the opinion of the writer that future study will prove that this phenomenon may occur in other species of *Dictyuchus*, and perhaps even in other members of the family.

In conclusion the writer wishes to express his thanks to Dr. Roland Thaxter, under whose kindly supervision these observations were made.

Laboratory of Cryptogamic Botany
Harvard University
Cambridge, Mass.

LITERATURE CITED

1. Atkinson, G. F., Some problems in the evolution of the lower fungi. Ann. Mycol. 7:441–472. *figs. 20.* 1909.
2. Butler, E. J., An account of the genus *Pythium* and some Chytridiaceae. Mem. Dept. Agric. India, Bot. Series 1:1–160. *pls. 1–10.* 1907.
3. Cornu, M., Monographie des Saprolegniees. Ann. Sci. Nat. V. 15:5–198. *pls. 1–7.* 1872.

4. FISCHER, A., Saprolegniineae, in Rabenhorst's Kryptogamenflora, 1^4:310–383. *figs. 13.* 1892.
5. HUMPHREY, J. E., The Saprolegniaceae of the United States. Trans. Amer. Phil. Soc. 17:1–148. *pls. 7.* 1893.
6. LEITGEB, H., Neue Saprolegnieen. Prings. Jahrb. Wiss. Bot. 7:357–387. *pls. 22–24.* 1869.
7. LINDSTEDT, K., Synopsis der Saprolegnniaceen und Beobachtungen über einiger Arten. Berlin. pp. 69. *pl. 4.* 1872.
8. MINDEN, M. VON, Saprolegniineae, in Kryptogamenflora der Mark Brandenburg, 5^4:209–600. 1912.
9. PIETERS, A. J., New species of *Achlya* and *Saprolegnia*. BOT. GAZ. 60: 483–490. *pl. 1.* 1915.
10. TIESENHAUSEN, M., Beiträge zur Kenntnis der Wasserpilze der Schweiz. Arch. Hydrobiol. Planktonk., Biol. Sta. Plön. 7^2:261–308. 1912.
11. WESTON, W. H., Observations on an *Achlya* lacking sexual reproduction. Amer. Jour. Bot. 4:354–367. *pl. 18.* 1917.
12. ZOPF, W., Über eine Saprolegniee mit einer Art von Erysipheenähnlicher Fruchtbildung. Beiträge Physiol. Morphol. Nied. Org. 3:48–59. *pls. 2, 3.* 1893.

EXPLANATION OF PLATE XXIII

The figures were drawn from living material at the level of the stage with the aid of an Abbé camera lucida. The approximate magnification of the combination of lenses used is given in each case, but applies to the original figures, which have been reduced to about two-thirds of their diameter in reproduction.

FIG. 1.—Two successively formed sporangia, the terminal empty, showing "cell-net" structure; second still containing spores; ×550.

FIG. 2.—Sporangium developed from starved hypha, showing incomplete uniting of spore walls; ×550.

FIGS. 3–7.—Stages in emergence of zoospore from sporangiospore; ×1400.

FIG. 8.—Zoospore after liberation; oblique later view; ×1400.

FIG. 9.—Same just starting to swim away; side view; ×1400.

FIG. 10.—Encysted zoospore (cystospore); ×1400.

FIGS. 11–13.—Stages in emergence of zoospore from cystospore; ×1400.

FIG. 14.—Zoospore just after emerging; side view; ×1400.

FIG. 15.—Same just starting to swim away; looking down on grooved surface; ×1400.

FIGS. 16–17.—Cystospore germinating by formation of hypha; ×1400.

FIG. 18.—Germination of sporangiospores *in situ* by hyphae; ×550.

FIG. 19.—Separation of sporangium from its hypha by rupture of sporangium wall at base; ×1400.